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PATENT

Attorney Docket No. 2307U-031212US

By _____

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:)

S. Baekkeskov et al.)

Serial No.: 08/452,053)

Filed: May 25, 1995)

For: METHODS FOR THE DIAGNOSIS)
AND TREATMENT OF DIABETES)

Examiner: D. Wortman

Art Unit: 1815

DECLARATION OF
STEINUNN BAEKKESKOV
Under 37 C.F.R §1.132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Steinunn Baekkeskov, a co-inventor of the above-referenced application, state as follows.

(1) I am an associate professor of Medicine and Microbiology/Immunology at the University of California, the assignee of the above-captioned application. A copy of my curriculum vitae is attached as Exhibit A.

(2) I have reviewed the application and followed the prosecution history thereof.

(3) I understand the Examiner has cited Baekkeskov et al., Nature 298, 167-169 (1982); Christie et al., Diabetologia 3, 597-602 (1988) and Baekkeskov et al., Diabetes 38, 1133-1141 (1989).

(4) The above references discuss detection of IDDM autoantibodies using a crude detergent extracts of GAD. The extract was prepared by treating ³⁵S-methionine labelled islets with Triton®-X-114 and centrifuging at 100,000 g. The resulting supernatant was then subjected to temperature phase separation,

resulting in partial separation of the 64 kD autoantigen together with other amphophilic proteins. The degree of purity of the 64 kD antigen prepared by this method was less than 1% as determined by 2D gel electrophoresis and either silver staining or autoradiography and densitometry (Baekkeskov et al., *Diabetes* 38, 1133-1141 (1989)).

(5) In my opinion, it would have been extremely difficult to purify the 64 kD antigen to the extent to at least 50% or 99% pure from the natural source of β -pancreatic islet cells at the priority date of the application. This difficulty is based on the following reasoning.

One difficulty limiting purification was the small quantities of pancreatic islet cells available and the fact that of this small amount of material, that 64 kD antigen was known to constitute less than 0.1% of total islet proteins (Baekkeskov et al., *Diabetes* 38, 1133-1141 (1989)).

A second difficulty was the absence of a simple and specific assay by which the yield of antigen could be assessed at each step in a purification procedure. The yield could be assessed only by immunoprecipitation of radioactive proteins followed by gel analysis and autoradiography. No other technique was sensitive enough to detect the minute amounts of protein obtained from islets. As well as being tedious, this assay procedure consumes much of the sample sought to be purified.

A third difficulty was the need to achieve purification without impairing the conformation of the 64 kD antigen which was expected to be required for subsequent use of the antigen as an assay reagent. The need to preserve conformation would have restricted the choice of solvents from the repertoire generally employed in protein purification (e.g., use of SDS or organic solvents would denature the antigen).

A fourth difficult was the heterogenous nature of the 64 kD antigen. As noted in the background section of the present application, the 64 kD antigen exists in a number of forms differing with respect to subcellular location, hydrophobicity and charge/mobility on SDS-gels (see specification at p. 4).

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Thus, different conditions might have been required to have attempted to purify each of the different forms. Without attempting to preserve each of the different forms throughout purification, there would have been no assurance that what was being purified was the appropriate form of the 64 kD antigen for detecting autoantibodies.

For these reasons, I do not believe that I or other skilled practitioners would have succeeded in obtaining the 64 kD antigen in at least 50% or 99% pure form suitable for use in diagnostic assays without the insight that the 64 kD autoantigen was GAD, which allowed purification from more abundant sources such as brain or a recombinant expression system.

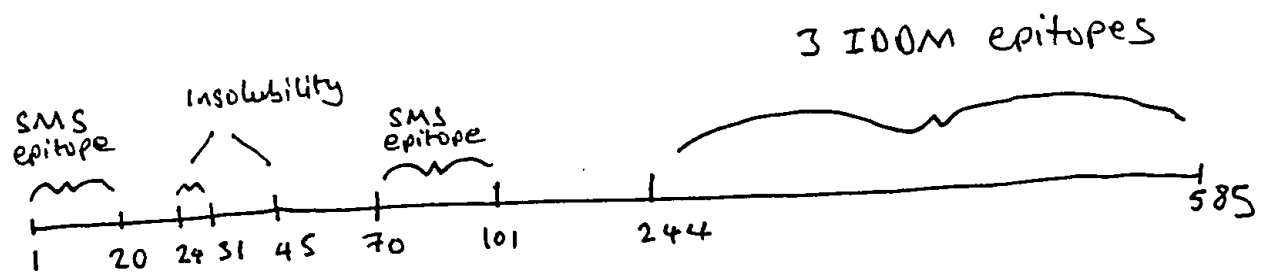
(6) I spent a year of my scientific life (1984-85) attempting to purify minute amounts of radioactive 64 kD antigen from 26 human islet cell preparation, but did not obtain sufficient quantities to obtain a sequence, even though I collaborated with Drs. Hunkapiller and Hood, who had the best microsequencing facility in the world at the time and had pioneered the microsequencing technique. I am also aware of several futile attempts to purify the protein from islet cells by other groups.

(7) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/5/97

By: Steinunn Baekkeskov
Steinunn Baekkeskov

Encl.: curriculum vitae



Map GAD65 showing residues conferring insolubility, Stiff man Syndrome (SMS) epitopes, and Insulin Dependent Diabetes Mellitus (IDDM) epitopes.